EXPRESS MAIL CERTIFICATE

Date 4/22/02 Label No. EV 028721855-US

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B.W. LEE B.W. Lee
Name (Print) Signature

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Customer No.: 07278
PATENT TRADEMARK OFFICE

Docket No: 9373/1H812-US3

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Zhen-Gang WANG et al.

Serial No.: 10/016,668 Art Unit: 1642

Confirmation No.: 3843

Filed: 10/26/01 Examiner: To be assigned

For: GENE RECOMBINATION AND HYBRID PROTEIN DEVELOPMENT

PRELIMINARY AMENDMENT UNDER 37 C.F.R. § 1.111

Hon. Commissioner of Patents and Trademarks
Washington, DC 20231

Sir:

In accordance with Rule 111 of the Rules of Practice, please enter the following amendment and consider the accompanying remarks before examining the above-captioned patent application. The amendments are made pursuant to the requirements of Rule 121 of the Rules of Practice. Accordingly, Applicants are

submitting herewith at Exhibit 1, a marked up copy of each amended paragraph in the specification showing all changes relative to the specification as originally filed. Applicants also submit herewith a Submission of Substitute Drawings transmittal, including Exhibits A-B with substitute **FIGS. 2, 3, 4C, 4D, 7, 8, 9,10, 12, 19 and 23** for entry in this application.

It is believed that no fees are required for this amendment. However, should the U.S. Patent and Trademark Office determine that any additional fees are required or that a refund is owed for this application, the Commissioner is hereby authorized and requested to charge the required fee(s) and/or credit the refund(s) owed to Deposit Account No. 04-0100.

Please amend the application as follows:

IN THE DRAWINGS:

Please delete **FIGS. 2, 3, 4C, 4D, 7, 8, 9, 10, 12, 19 and 23** as originally filed for this application and enter, in their place, substitute **FIGS. 2, 3, 4C, 4D, 7, 8, 9, 10, 12, 19 and 23** attached at Exhibit Tab A of the accompanying Submission of Substitute Drawings.

IN THE SPECIFICATION:

Please amend the specification as follows:

Amend page 1at line 11 of the specification by inserting the following new paragraph which reads as follows:

- - Work leading to this invention was supported by Grant No. N00014-96-1-0340 awarded by the United States Navy. The United States government may have certain rights to this invention, pursuant to the term of that grant. - -

Amend the paragraph at lines 24-27 on page 15 of the specification, as indicated in the accompanying Exhibit 1, so that the paragraph reads as follows:

- - **FIG. 3** is a gene alignment for β-lactamase-like genes, (1) *Enterobacter cloacae,* **SEQ. ID NO.1**; (2) *Citrobacter freundii,* **SEQ. ID NO. 2**; (3) *Yersinia enterocolitica* , **SEQ. ID NO.3**; *and* (4) *Klebsiella pneumonia,* **SEQ. ID NO.4**. SWISPROT or TrEMBL accession numbers for the protein sequences and GenBank accession numbers for the DNA sequences are given. - -

Amend the paragraph at lines 1-3 on page 17 of the specification, as indicated in the accompanying Exhibit 1, so that the paragraph reads as follows:

- - **FIG. 7** is an example of an *in vitro* method of overlap extension reassembly, targeting identified crossover locations. The appropriate fragments may be obtained by split-pool synthesis. In **FIG. 7**, part (A), all possible recombinants are prepared by crossover at positions 1 and 2. In **FIG 7**, part (B), the recombinants can be prepared by assembly of synthetic fragments containing the crossover positions. This example requires fragments (plus end primers). - -

Amend the paragraph at lines 4-7 on page 17 of the specification, as indicated in the accompanying Exhibit 1, so that the paragraph reads as follows:

- - **FIG. 8**, part (A), shows a fragment reassembly method using a parental template. The synthetic fragments are extended against a parent template strand and the gaps are repaired. In **FIG. 8**, part (B), the resulting products are subjected to heteroduplex recombination (Volkov *et al., Nucl. Acids Res.*, 27:18 (1999))to create libraries of genes within regions of non-identity. More complexity can be introduced by the addition of more fragments during template assembly. - -

Amend the paragraph at lines 8-9 on page 17 of the specification, as indicated in the accompanying Exhibit 1, so that the paragraph reads as follows:

- - **FIG. 9** shows the preparation of gene fragments prepared by PCR with primers directed to regions targeted for crossovers. In **FIG. 9**, part (A), the fragments are prepared by PCR with primers. The PCR reactions are performed with primers 1 + 2, 3 + 4 and 5 + 6. The method is repeated for the other parents.- -

Amend the paragraph at lines 10-11 on page 17 of the specification, as indicated in the accompanying Exhibit 1, so that the paragraph reads as follows:

- - **FIG. 10** shows recombination directed to specific sites using crossover primers in DNA shuffling. In **FIG. 10**, part (A), crossover primers designed to have crossovers at designated positions (2 primers for each position) are prepared. In **FIG. 10**, part (B), the parent genes are fragmented and reassembled, utilizing PCR

methods, in the presence of the crossover primers to promote recombination at designated positions.- -

Amend the paragraph at lines 14-15 on page 17 of the specification, as indicated in the accompanying Exhibit 1, so that the paragraph reads as follows:

- - **FIG. 12** is a flow diagram illustrating one embodiment of a recombinant search algorithm of the invention, based upon sequence identity. In **FIG. 12**, part (1), the parent sequences are aligned with the template structure. In **FIG 12**, part (2), all possible crossover points are determined according to a sequence identity algorithm . In **FIG. 12**, part (3), the coupling matrix is calculated. In **FIG. 12**, part (4), a start parent is picked at random and copied to the offspring until a possible cut point is reached. In **FIG. 12**, part (5), a random number is picked, and if the number is less than *p*, a random new parent is copied until the next cut point is reached. In **FIG. 12**, part (6), the crossover disruption of the offspring gene is determined. - -

Amend the paragraph at lines 16-26 on page 18 of the specification, as indicated in the accompanying Exhibit 1, so that the paragraph reads as follows:

- - **FIG. 19** is a schematic demonstrating the utility of a contact map in identifying compact units of substructure. A representative contact map is on the left. The graph on the right is a statistical study of the average length of contiguous residues that can fold into a sphere of the indicated diameter (Gilbert 1998). This information can be used in the following way. If a 15-residue segment can fold into a sphere with a diameter of 21 angstroms, then this segment could be considered as

being of average compactness. However, if a 20-residue segment can fold into a sphere of 21 angstroms, this is considered as having a significantly above-average compactness. This is visualized on the contact map as a triangle on the diagonal formed by the cut points required to generate the segment. If the segment fits into a sphere of the specified diameter, then the triangle will be entirely white (interacting). The contact map shows residues that are distant (black) and residues that are close (white). If a given segment, ▬▬, folds an above average number of residues into a given sphere size, then it is compact.- -

Please amend the specification at page 122, line 21, as indicated in the accompanying Exhibit 1, so that the paragraph reads as follows:

- - To test our ability to predict crossover locations, we designed experiments to recombine fragments of two beta-lactamases, TEM-1 and PSE-4, using the SOEing procedure to piece together fragments by PCR (Horton, R. M., (1995) *Mol. Biotech.* 3, 93-99). While the proteins in this example have only 40% amino acid sequence identity, they share similar structures (TEM-1 , SEQ ID NO: 5; and PSE-4 SEQ ID NO: 6) (Jelsch, C., Mourey, L., Masson, J. M., & Samama, J. P., (1993) *Proteins* 16, 364; Lim, D., Sanschagrin, F., Passmore, L., De Castro, L., Levesque, R. L., & Strynadna N. C. J., (2001) *Biochemistry* 40, 395). - -

Please delete pages 126 to 155 as originally filed for this application and enter, in their place, substitute pages 126-156 attached at Exhibit 2 of the accompanying amendment.

REMARKS

The specification on page 1 for this application has been amended solely to introduce acknowledgment of Federal Support of work leading to the invention contained in this application. Thus, no new matter has been introduced by this amendment.

The specification and Figures for this application have been amended in order to comply with the requirements for drawings and application under 37 C.F.R. § 1.75(h); §1.84(o); § 1.84(e) and § 1.821-1.825.

In particular, the drawings have been amended to remove excessive text, and darken and/or improve print quality. The specification has also been amended to reflect these changes to the drawings. In particular, the Brief Description of the drawings has been amended to incorporate the description originally provided as part of the drawings. In addition, the specification has been amended to incorporate appropriate sequence identifiers from the accompanying Sequence Listing. Also, in order to comply with 37 C.F.R. §1.75 (h) substitute pages have been submitted in order to have the claims commence on a separate sheet of paper.

The above made amendments do not introduce new matter to the present application. It is believed that this preliminary amendment does not unduly interfere

with the preparation of the first Office Action for this Application. Accordingly, entry of these amendments is respectfully requested.

Respectfully submitted,

Dated: April 22, 2002

Samuel S. Woodley, Ph.D.
Reg. No. 43,287
Agent for Applicants

DARBY & DARBY, P.C.
805 Third Avenue
New York, N.Y. 10022
Phone (212) 527-7700

EXHIBIT 1: AMENDMENTS TO U.S. PATENT APPLICATION SERIAL NO. 09/863,765 (ATTORNEY DOCKET NO. 9373/1H812US3)

SUBMITTED PURSUANT TO 37 C.F.R. § 1.121(b)(1)(iii)

Amend page 1at line 11 of the specification by inserting the following new paragraph which reads as follows:

Work leading to this invention was supported by Grant No. N00014-96-1-0340 awarded by the United States Navy. The United States government may have certain rights to this invention, pursuant to the term of that grant.

Amend the paragraph at lines 24-27 on page 15 of the specification so that the paragraph reads as follows:

FIG. 3 is a gene alignment for β-lactamase-like genes, (1) *Enterobacter cloacae,* **SEQ. ID NO.1,**; (2) *Citrobacter freundii,* **SEQ. ID NO.2**; (3) *Yersinia enterocolitica* , **SEQ. ID NO.3**; *and* (4) *Klebsiella pneumonia*, **SEQ. ID NO.4**. SWISPROT or TrEMBL accession numbers for the protein sequences and GenBank accession numbers for the DNA sequences are given.

The paragraph at lines 1-3 on page 17 of the specification should be amended as follows:

FIG. 7 is an example of an *in vitro* method of overlap extension reassembly, targeting identified crossover locations. The appropriate fragments may be obtained by split-pool synthesis. In **FIG. 7**, part (A), all possible recombinants are

prepared by crossover at positions 1 and 2. In **FIG 7**, part (B), the recombinants can be prepared by assembly of synthetic fragments containing the crossover positions. This example requires fragments (plus end primers).

The paragraph at lines 4-7 on page 17 of the specification should be amended as follows:

FIG. 8, part (A), [**A**]shows a fragment reassembly method using a parental template. The synthetic fragments are extended against a parent template strand and the gaps are repaired. In **FIG. 8**, part (B), t[T]he resulting products are subjected to heteroduplex recombination (Volkov *et al., Nucl. Acids Res.*, 27:18 (1999))to create libraries of genes within regions of non-identity. More complexity can be introduced by the addition of more fragments during template assembly.

The paragraph at lines 8-9 on page 17 of the specification should be amended as follows:

FIG. 9 shows the preparation of gene fragments prepared by PCR with primers directed to regions targeted for crossovers. In **FIG. 9**, part (A), the fragments are prepared by PCR with primers. The PCR reactions are performed with primers 1 + 2, 3 + 4 and 5 + 6. The method is repeated for the other parents.

The paragraph at lines 10-11 on page 17 of the specification should be amended as follows:

FIG. 10 shows recombination directed to specific sites using crossover primers in DNA shuffling. In **FIG. 10**, part (A), crossover primers designed to have crossovers at designated positions (2 primers for each position) are prepared. In **FIG. 10**, part (B), the parent genes are fragmented and reassembled , utilizing PCR methods, in the presence of the crossover primers to promote recombination at designated positions.

The paragraph at lines 14-15 on page 17 of the specification should be amended as follows:

FIG. 12 is a flow diagram illustrating one embodiment of a recombinant search algorithm of the invention, based upon sequence identity. In **FIG. 12**, part (1), the parent sequences are aligned with the template structure. In **FIG 12**, part (2), all possible crossover points are determined according to a sequence identity algorithm. In **FIG. 12**, part (3), the coupling matrix is calculated. In **FIG. 12**, part (4), a start parent is picked at random and copied to the offspring until a possible cut point is reached. In **FIG. 12**, part (5), a random number is picked, and if the number is less than p, a random new parent is copied until the next cut point is reached. In **FIG. 12**, part (6), the crossover disruption of the offspring gene is determined.

The paragraph at line 16-26 on page 26 of the specification should be amended as follows:

FIG. 19 is a schematic demonstrating the utility of a contact map in identifying compact units of substructure. A representative contact map is on the left. The graph on the right is a statistical study of the average length of contiguous residues that can fold into a sphere of the indicated diameter (Gilbert 1998). This information can be used in the following way. If a 15-residue segment can fold into a sphere with a diameter of 21 angstroms, then this segment could be considered as being of average compactness. However, if a 20-residue segment can fold into a sphere of 21 angstroms, this is considered as having a significantly above-average compactness. This is visualized on the contact map as a triangle on the diagonal formed by the cut points required to generate the segment. If the segment fits into a sphere of the specified diameter, then the triangle will be entirely white (interacting). The contact map shows residues that are distant (black) and residues that are close (white). If a given segment, [redacted], folds an above average number of residues into a given sphere size, then it is compact.

Please amend the specification at page 122, line 21, as indicated in the accompanying Exhibit 1, so that the paragraph reads as follows:

To test our ability to predict crossover locations, we designed experiments to recombine fragments of two beta-lactamases, TEM-1 and PSE-4, using

the SOEing procedure to piece together fragments by PCR (Horton, R. M., (1995) *Mol. Biotech.* 3, 93-99). While the proteins in this example have only 40% amino acid sequence identity, they share similar structures (TEM-1 , SEQ ID NO: 5; and PSE-4 SEQ ID NO: 6) (Jelsch, C., Mourey, L., Masson, J. M., & Samama, J. P., (1993) *Proteins* 16, 364; Lim, D., Sanschagrin, F., Passmore, L., De Castro, L., Levesque, R. L., & Strynadna N. C. J., (2001) *Biochemistry* 40, 395).

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B.W. LEE B.W. Lee
Name (Print) Signature

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Customer No.: 07278
PATENT TRADEMARK OFFICE

Docket No: 9373/1H812-US3

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Zhen-Gang WANG et al.

Serial No.: 10/016,668 Art Unit: 1642

Confirmation No.: 3843

Filed: 10/26/2001 Examiner: To be assigned

For: GENE RECOMBINATION AND HYBRID PROTEIN DEVELOPMENT

SUBMISSION OF SUBSTITUTE DRAWINGS

Hon. Commissioner of Patents and Trademarks
Washington, DC 20231

Sir:

In response to the Notice to File Missing Parts (hereinafter referred to as the "Notice") mailed on February 22, 2002 for the above-captioned patent application and in accordance with 37 C.F.R. 1.821-1.825 and 1.84, Applicants respectfully request authorization to amend the drawings as filed for this application.

The Notice indicates that the drawings as filed have been rejected under 37 C.F.R. § 1.84(e) because the previously submitted drawings are not electronically reproducible. In particular, Applicant understand that certain drawings submitted with the application as filed were too light to be electronically reproduced. In order to be fully responsive to the Notice, substitute drawings, which are darker and/ or of clearer print quality, are submitted herewith that are believed to be fully compliant with the requirements of 37 C.F.R. § 1.84(e). Specifically, darker and/or clearer print quality substitute drawings for FIGS. 2, 4C, 4D, 7, 8, 10, and 12, are submitted herewith at Exhibit Tab A. The drawings have been changed only to darken the drawings and do not introduce new matter to the application.

In addition, Applicant understand that certain drawings failed to comply with the requirements of 37 C.F.R. §1.84(o) in that they contain excessive text. In order to comply with 37 C.F.R. §1.84(o), substitute drawings without the excessive text are submitted herewith.

The Notice also indicates that the drawings as filed have been rejected under 37 C.F.R. 1.821-1.825. In order to be fully responsive to the Notice, a substitute drawing is submitted herewith that is believed to be fully compliant with the requirement of 37 C.F.R. 1.821-1.825 and 37 C.F.R. 1.84(O). The amino acid sequences of listed in **FIG. 3** have been given SEQ ID NOS 1-4. (In addition, the amino acid "Sequence Listings" are submitted herein with an amendment directing entry.)

Substitute drawings for FIGS. 3, 7, 8, 9-10, 12, 19 and 23, are submitted herewith at Exhibit Tab A. Redlined copies of these drawings are also attached hereto, at Exhibit Tab B. These redlined copies demonstrate that the drawings have been changed only to remove excessive text and do not introduce new matter to the application.

Reconsideration of the drawings for this application, in view of the substitute Figures submitted herewith is respectfully requested.

Respectfully submitted,

Dated: April 22, 2002

Samuel S. Woodley, Ph.D.
Reg. No. 43,287
Agent for Applicants

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New York, N.Y. 10022
Phone (212) 527-7700

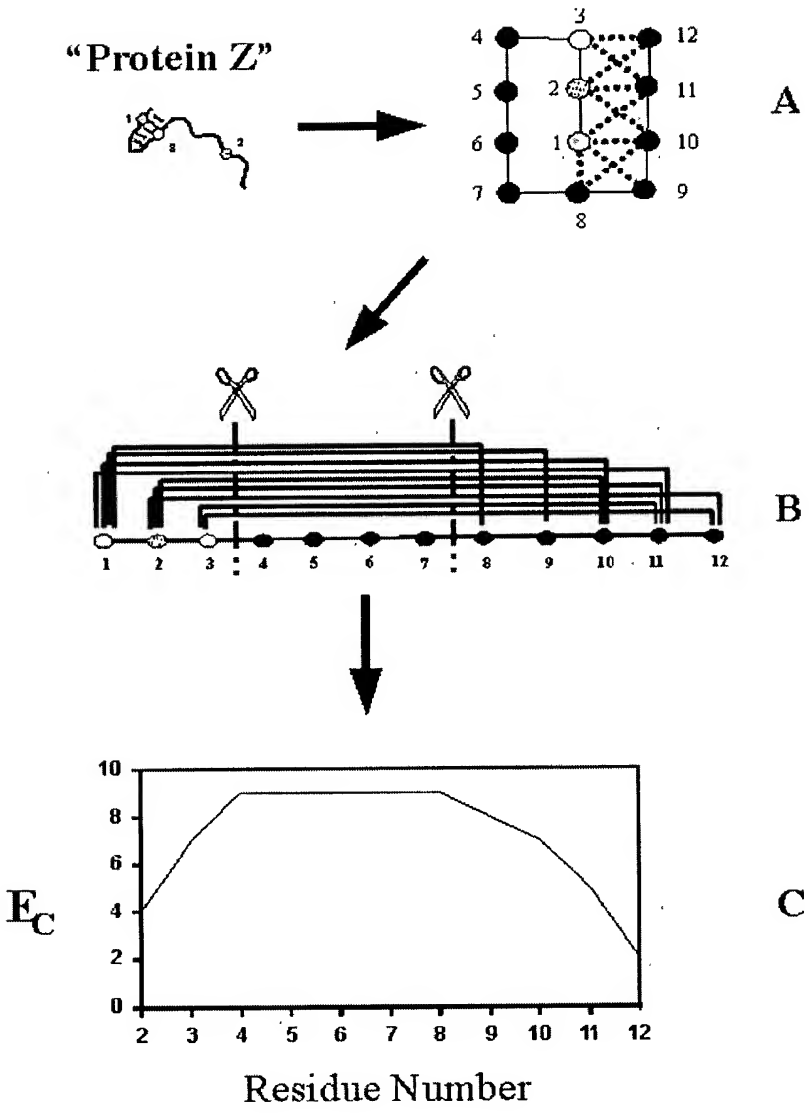
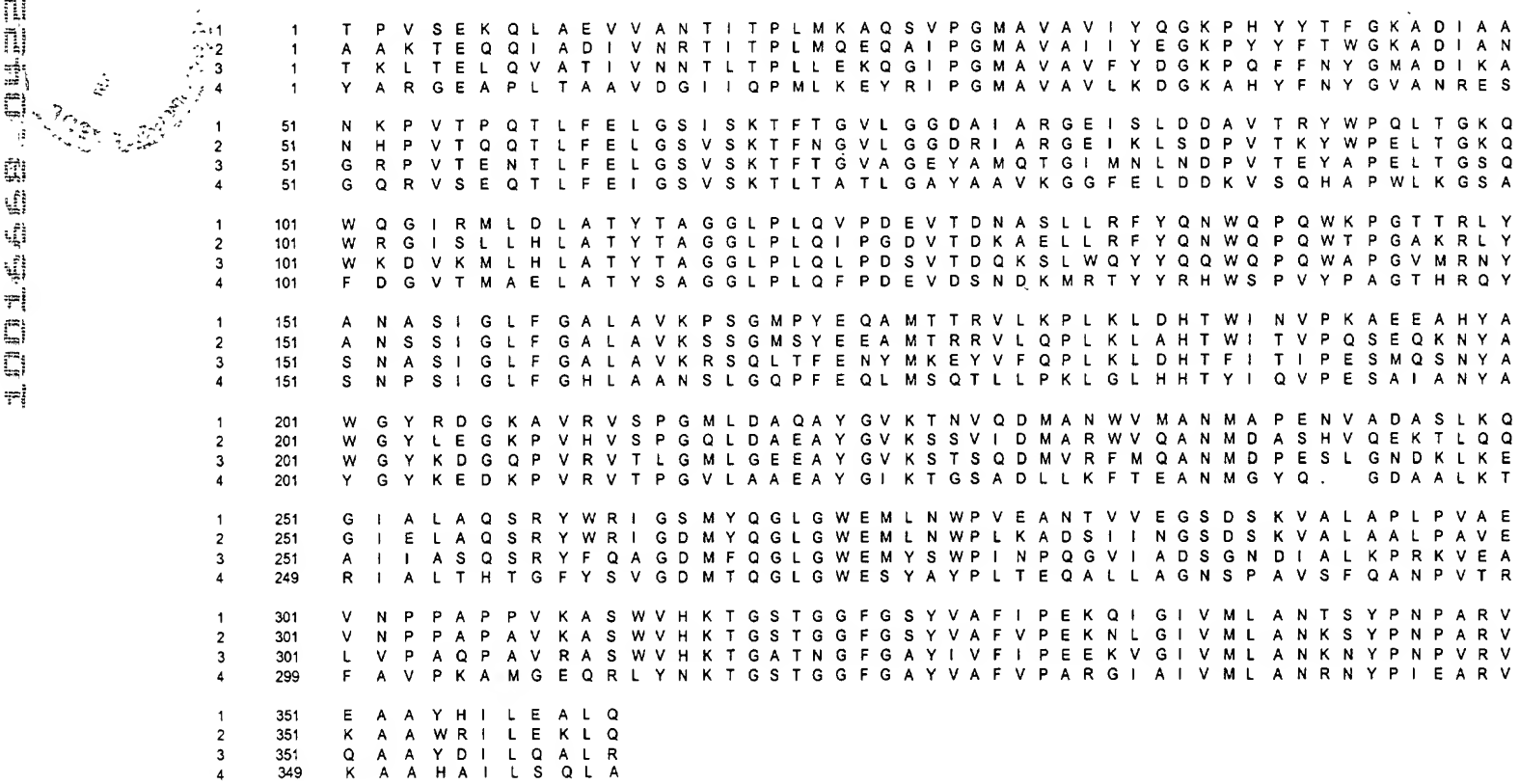


FIG. 2



1	Enterbacter cloacae	P05364 ~~(X03866)~~ (SEQ ID NO:1)
2	Citrobacter freundii	P05193 ~~(X07274)~~ (SEQ ID NO:2)
3	Yersinia enterocolitica	P45460 ~~(X63149)~~ (SEQ ID NO:3)
4	Klebsiella pneumoniae	Q48437 ~~(X77455)~~ (SEQ ID NO:4)

FIG. 3

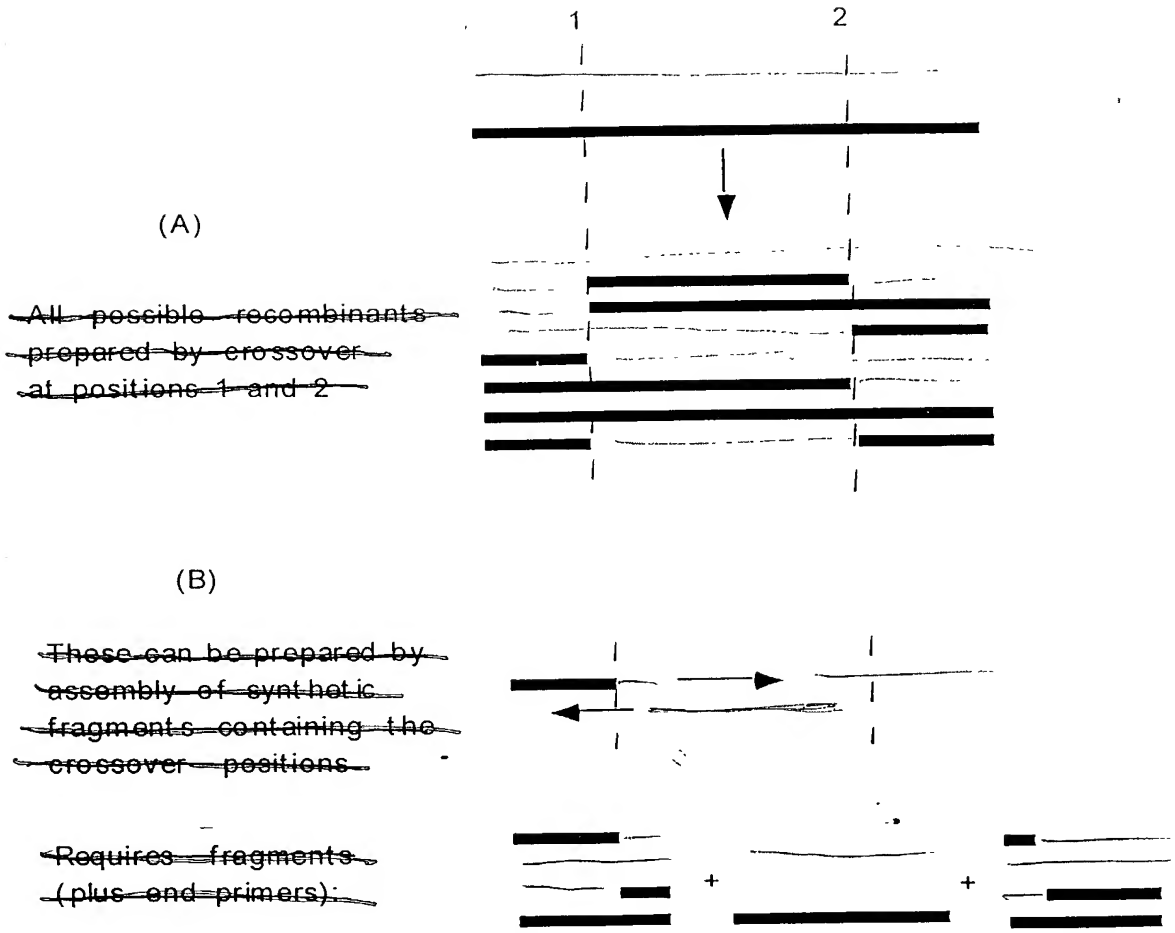


FIG. 7

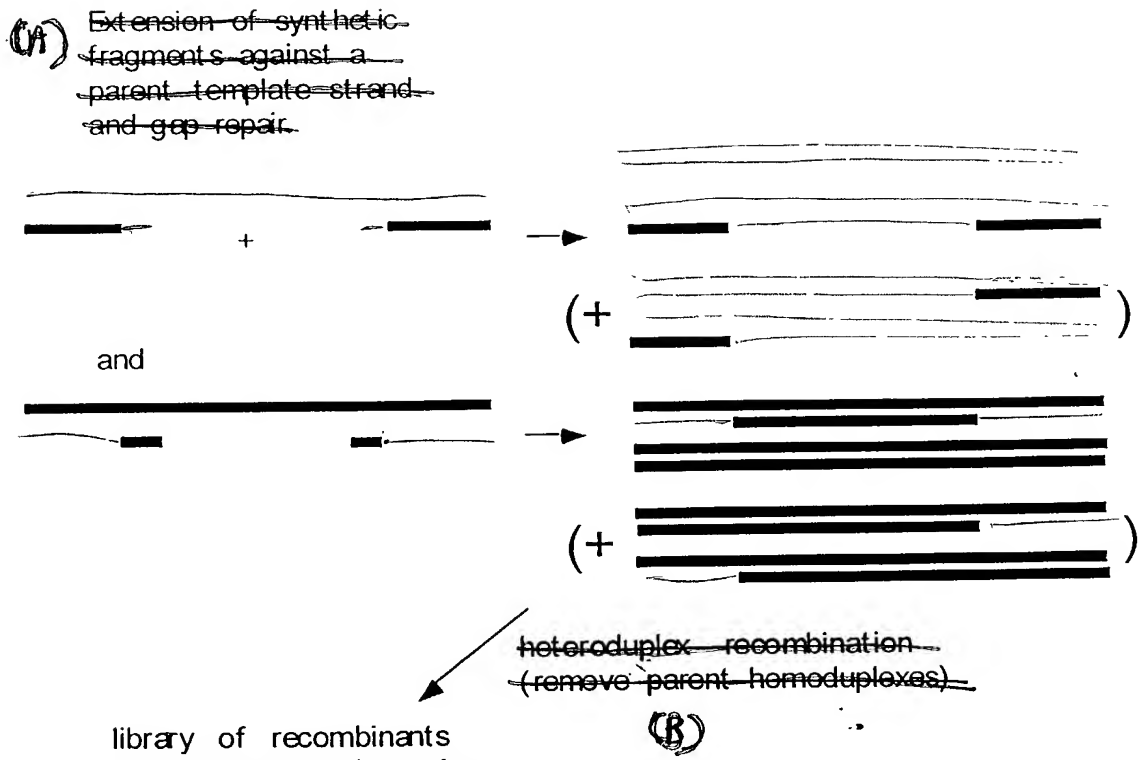
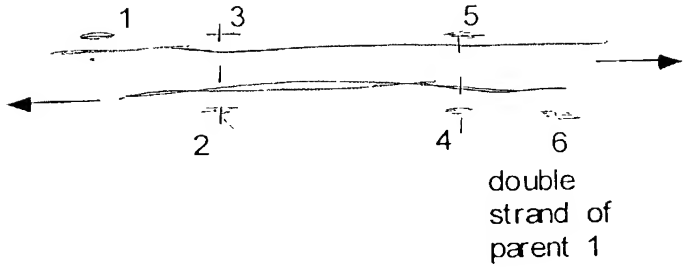


FIG. 8

(A)

~~Prepare the fragments by PCR with primers: perform reactions with primers 1+2, 3+4 and 5+6,~~

~~and do same for other parent(s).~~



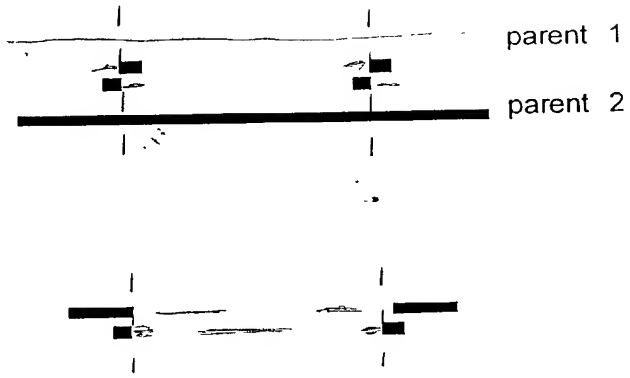
(B)

Reassemble fragments in a pool, by PCR with 1+ 6

FIG. 9

(A)

~~Prepare crossover primers designed to have crossovers at designated positions (2 primers for each position).~~



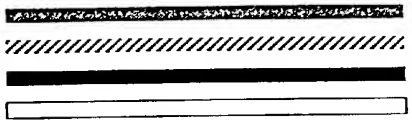
(B)

~~Fragment parent genes and PCR reassemble in the presence of the crossover primers to promote recombination at designated positions~~

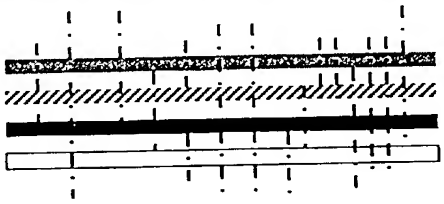
FIG. 10

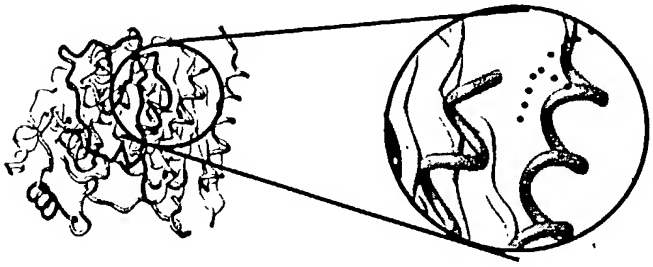
Recombinant search algorithm

1. Align parent sequences with template structure



2. Determine all possible crossover points according to sequence identity algorithm

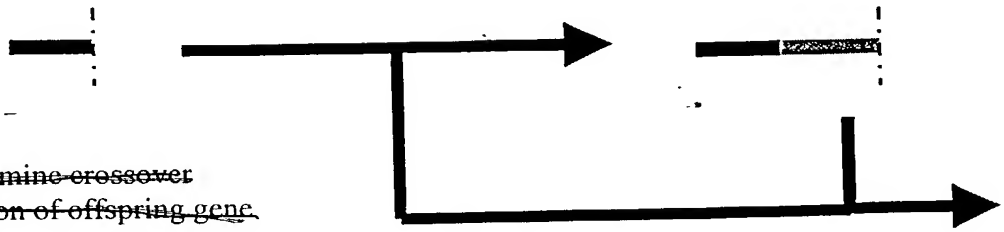




3. Calculate coupling matrix

4. Pick start parent at random and copy to offspring until a possible cut point is reached.

5. Pick random number, if less than p, copy random new parent until next cut point is reached.



6. Determine crossover disruption of offspring gene.

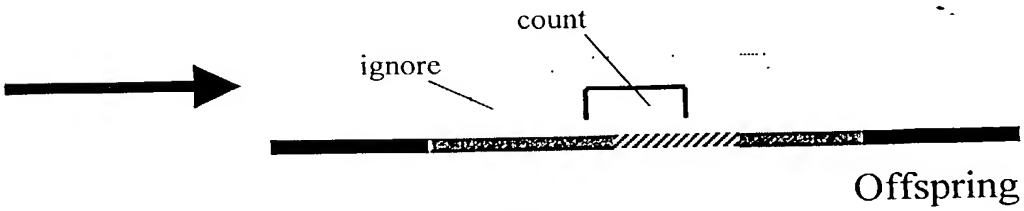


FIG. 12

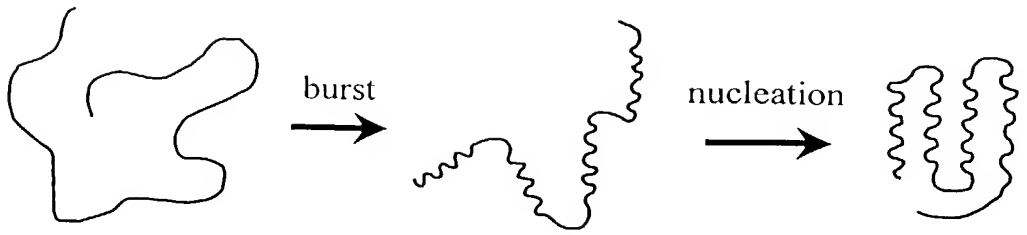


FIG. 18

~~The contact map shows residues that are distant (black) and residues that are close (white). If a given segment, [redacted], folds an above average number of residues into a given sphere size, then it is compact.~~

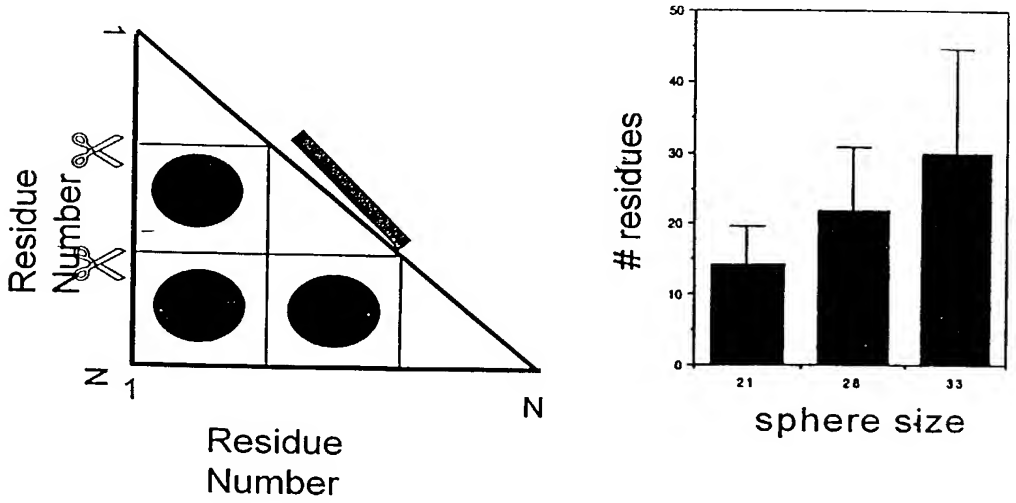


FIG. 19

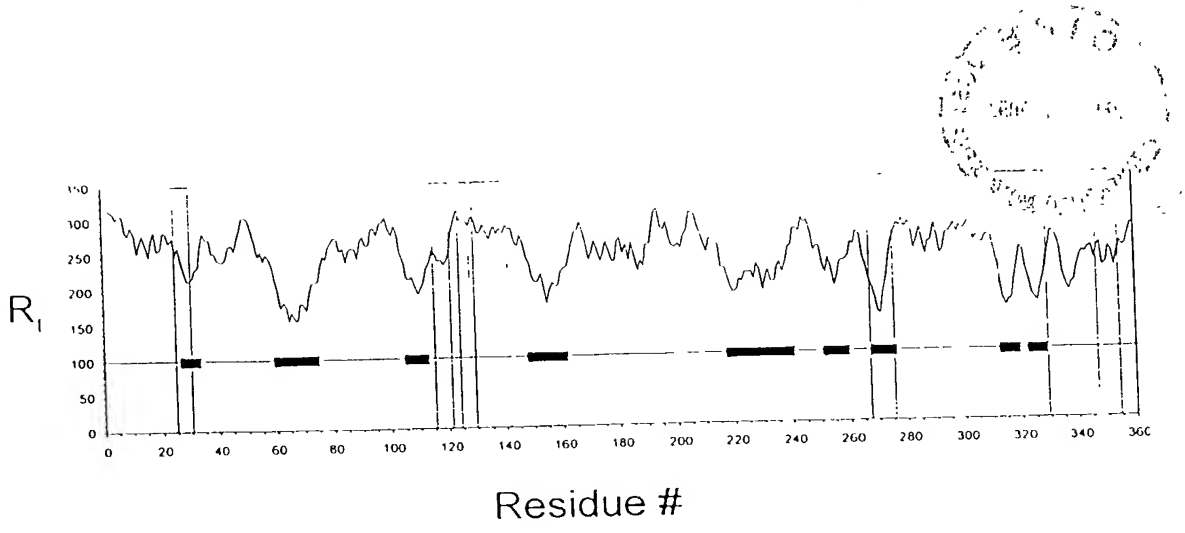
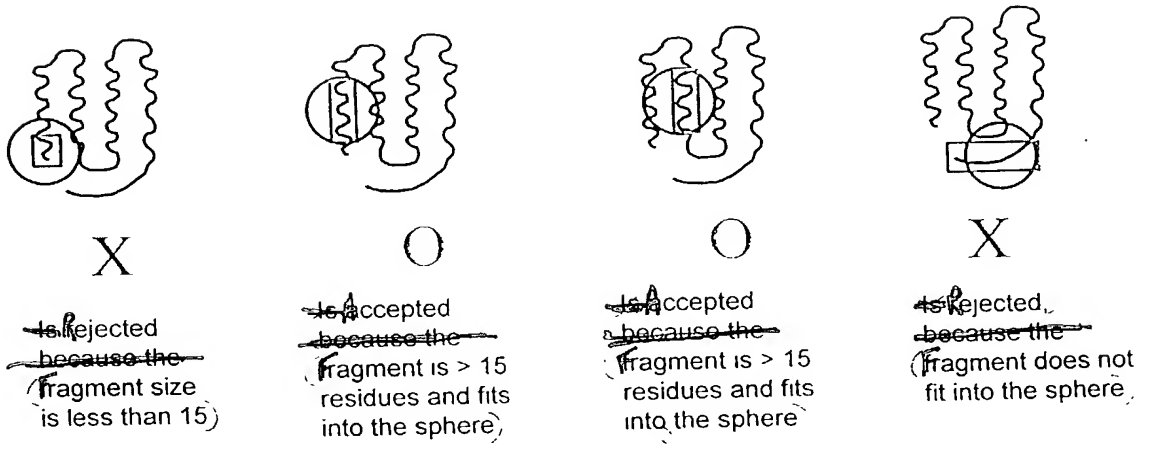


FIG. 22



(1) Pick a sphere size (21 angstroms, like Go-Gilbert) and a disruption threshold; (2) Scan protein using segments at least the average number of residues for that sphere size or greater (e.g., >15 for 21 angstrom sphere); 3) Check the disruption of all the compact fragments identified in step 2. If the fragment has a disruption above a threshold value, keep it; otherwise, throw it out; 4) If the compact unit is disruptive, increment the schema disruption measure for all of the residues in the fragment by one. This indicates that crossovers within the fragment are disfavored.

FIG. 23